

The sulphated polysaccharide compounds from green algae (*Ulva lactuca* L) as a potential natural anti-inflammatory agent based on molecular docking study targeting cyclooxygenase-2 receptor

Dwi Utami^{1*}, Rezza Wahyudi², Wahyu Widyaningsih³

¹Departement of Analytical and Medicinal Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl Prof. Dr. Soepomo, S.H, Warungboto, Yogyakarta, Indonesia

²Pharmacy Study Programme, Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl Prof. Dr. Soepomo, S.H, Warungboto, Yogyakarta, Indonesia

³Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl. Prof. Dr. Soepomo, S.H, Warungboto, Yogyakarta 55164

Submitted: 15-02-2023

Reviewed: 02-04-2023

Accepted: 24-07-2023

ABSTRACT

The rare sulphated polysaccharides of green algae have been explored of several activities such as antioxidant, anti-inflammation, anti-bacterial, and anti-virus. *Ulva lactuca* L is one of the majoring algae species in Indonesia that promising to be explored as natural anti-inflammation in the inflammation disorders treatment. The purpose of this study was to conduct an in-silico test of anti-inflammatory potency of sulphated polysaccharide chemical constituent of green algae (*Ulva lactuca* L) against the cyclooxygenase-2 enzyme. The methods used were the preparation of a protein structure database cyclooxygenase-2 enzyme (4COX), protein preparation using the Biovia Discovery Studio application, molecular docking simulation of sulphated polysaccharide compounds on proteins using the Autodock 4.0 application and visualization using Ligpot+ v2.2. The results of docking sulphated polysaccharide compounds with the cyclooxygenase-2 enzyme, showed a best binding affinity energy of Gluconic acid ulvan -7.62 kcal/mol similar to the control drug sodium diclofenac (-7.81 kcal/mol), followed by Iduronic acid Ulvan -7.57 kcal/mol, Fucoidan (-6.11 kcal/mol), Alpha Carrageenan (-6.93 kcal/mol), and Lambda Carrageenan (-5.38 kcal/mol). In the conclusion based on the molecular docking result, the sulphated polysaccharide compounds in *Ulva lactuca* L are potential to be developed as natural antiinflammatory agent by *in vitro* and *in vivo* investigation.

Keywords: molecular docking, sulphated polysaccharide, anti-inflammatory, *Ulva lactuca* L, 4COX

*Corresponding author:

Dwi Utami

Departement of Analytical and Medicinal Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl Prof. Dr. Soepomo, S.H., Warungboto, Yogyakarta, Indonesia

Email: dwi.utami@pharm.uad.ac.id



INTRODUCTION

Ulva lactuca L is a green algae plant that is widely found in Indonesian waters. It around 782 marine algae which are composed of 196 types of green algae, 134 types of brown algae, and 452 types of red algae (Kepel et al., 2019). In recent decade, algae plants is used in the food industry, biomaterial, pharmacy, and cosmetics (Novianti et al., 2020). The content of the active ingredient in green algae that has been exploited is melatonin in ethanolic extract with antioxidant activities for heart ischemic or wound healing (Bernardi et al., 2015; Jin et al., 2018; Reiter et al., 2016). Another active substance with a large amount is a polysaccharide with a high fiber content for food industry and packaging materials (Tang et al., 2016; Tziveleka et al., 2018; Yu-Qing et al., 2016). One of the rare polysaccharide compounds that has not been widely explored is the sulphated polysaccharide that has been found in polar extract of green algae (Kidgell et al., 2019; Tabarsa et al., 2018).

Sulphated polysaccharides are a complex group of macromolecules with numerous biological activities, including antimicrobial, anti-allergy, anti-cancer, anti-coagulant, anti-inflammation, anti-oxidant, and anti-viral properties (Ahmadi et al., 2015; Kim et al., 2013; Li & Shah, 2014; Liu et al., 2022; Motoyama et al., 2018; Vasconcelos et al., 2013). Sulphated polysaccharide is found in all three major seaweed groups: brown, green, and red seaweeds. Fucans, such as fucoidan, sargassan, ascophyllan, and glucuronyloxyfucan, are brown sulphated polysaccharides; galactans, such as agar and carrageenan, are red sulphated polysaccharides. Sulphated heteropolysaccharides containing galactose, xylose, arabinose, mannose, glucuronic acid, or glucose are commonly used to make green SSPs (Jiao et al., 2011). Ulvan is primarily composed of sulphated rhamnose, glucuronic acid, iduronic acid, and xylose (Lakshmi et al., 2020). Due to its numerous physicochemical characteristics and significant biological activity, ulvan has been regarded as an appealing substance for use in food, pharmaceutical, agricultural, and medicinal applications (Cindana Mo'o et al., 2020).

In the anti-inflammatory activities point of view, Ulvan (or extracts containing ulvan) have been evaluated for their ability to modify the inflammatory response *in vitro* using macrophages (such as RAW264.7 cells) and *in vivo* using animal models (Kim et al., 2011; Peasura et al., 2016) in order to evaluate the effects of ulvan on human health. The impact of ulvan on inflammatory responses is frequently assessed *in vitro* by measuring the amounts of inflammatory cytokines released by macrophages, including tissue necrosis factor alpha (TNF-), interleukin (IL)-1, 4, 5, 6, 10, 12, 18, and prostaglandin E2 (PGE2) and nitric oxide (NO) (Leiro et al., 2010; Tabarsa et al., 2018). However, the comprehensive study of the anti-inflammatory molecular mechanism of ulvan based on its interaction with COX receptor as the well known anti-inflammatory receptor was limited.

Therefore, in this paper we initiate evaluation of the anti-inflammatory activities of green seeds of Indonesian waters from aqueous extract of *Ulva lactuca* L by *in vitro* and *in vivo* method, by molecular docking the potency of sulphated polysaccharides as antagonist agent in cyclooxygenase enzyme (COX-2) as the important enzyme in inflammation process. The molecular docking simulation result will predict the interaction of sulphated polysaccharides compound in *Ulva lactuca* L to the anti-inflammatory receptor. To the best of our knowledge, this is the first report on the interaction of sulfated polysaccharides from *Ulva lactuca* L with the COX receptor.

The molecular findings of the sulphated polysaccharide as a natural inhibitor against 4COX are examined in the current study using an *in-silico* approach. This work will contribute to our understanding of the molecular basis of action and the variety interaction of sulphated polysaccharides toward COX receptor. Later, sophisticated research could support sulphated polysaccharide as the natural anti-inflammatory agent and followed by *in vitro* and *in-vivo* examination of *Ulva lactuca* L in various pharmaceutical dosage form.

MATERIALS AND METHOD

Materials

The tool used in molecular docking study were a set of computers with specifications ASUS X441B CPU: AMD Dual Core (A6-9225, up to 3.0 GHz) RAM Memory: 4GB. Autodock 4.2.3 software from Script Research Institute, Biovia Discovey Studio (BDS), Avogadro, Pymol, SwissADME and pKCSM websites and Chem office 2010 software.

Methods

Preparation of proposed compounds

The 2D chemical structure of proposed compound were prepared by ChemDraw Profesional V.19 as shown in [Figure 2](#).

Protein and native ligand preparation

The 3D crystal structure data of the receptor used for the molecular docking analysis were obtained from the protein data bank (PDB) on the <http://rcsb.org/pdb/> site, with code pdb : 4COX. Receptors in the form of macromolecules are separated from other molecules such as water molecules and their natural ligands. The process of preparation of native ligand on protein tyrosinase was done by separating the ligands from other molecules such as water molecules, co-factors and their receptors. The separation was carried out using Biovia discovery studio software.

Docking method validation

The validation of the molecular docking method was carried out by the redocking method using native ligand. The validation process will give results that are closer to the experimental results if the Root-Mean-Square-Deviation (RMSD) value is less than 3 Å (angstrom) ([Jain & Nicholls, 2008](#)). The smaller the RMSD value indicates the position of the redocked ligand will resemble the crystallographic ligand coordinate and conformation ([Kontoyianni et al., 2004](#)).

Validation

The proposed ligands structure which were Gluconic acid ulvan, Iduronic acid ulvan, fucoidan, Alpha carrageenan, Iota carrageenan, Kappa carrageenan, and Lambda carrageenan were constructed using ChemDraw Profesional V.19 software. Then the geometry optimization was carried out using Avogadro software by adding hydrogen atoms to the test ligand structure and then energy minimization was carried out using the semi-empirical method (MM2) in Chem 3D software. This aimed to minimize the energy to obtain a more stable conformation. The optimization results are then converted into PDB files to be used in the molecular docking process in Autodock software.

Molecular docking of ligand-protein

Grid box parameter settings are performed using Autodock 4.2.6 The grid box coordinates are determined based on the native co-crystal ligand coordinates of the receptor file used during validation, then the bonding process between the test ligand and the receptor is carried out using autodock 4.2.3. The magnitudes of the X, Y, and Z axes used in grid boxes setting were (40,40,40) with coordinates (60.804, 44.603, 72.176) and a grid point spacing of 0.375 Å.

Docking results analysis and visualization

Determination of the conformation of the docking ligand (the best pose) was done by selecting the conformation of the ligand that has the lowest binding affinity. The docking results with the best poses are then analyzed using Biovia Discovery Studio. Parameters analyzed included binding affinity (ΔG), inhibition constant (KI), amino acid residues and the number of bond interactions formed. The Biovia discovery studio software was then used to visualize the results of the binding of the test compound to the protein by observing the presence of an interaction between the ligand and protein in 2D and PyMOL software 3D to visualize the surface area of the test ligand in the active site of 4COX receptor.

RESULT AND DISCUSSION

Preparation of 4COX protein and ligands

The COX's enzymatic activity involves the bis-oxygenation of arachidonic acid to PGG₂, which is then reduced to PGH₂ by the same protein in a peroxide reaction. Nonsteroidal anti-inflammatory

drugs (NSAIDs) act at the active site of cyclooxygenase and most inhibit both COX-1 and COX-2 with little specificity, resulting in serious side effects such as gastric lesions and renal toxicity. COX-2-selective inhibitors with potent anti-inflammatory activity in vivo and minimal gastrointestinal side effects have been identified (Kurumbail et al., 1996). The structure is divided into three distinct domains, as shown in Figure 1: an N-terminal epidermal growth factor (EGF) domain, a membrane-binding motif, and a C-terminal catalytic domain containing the cyclooxygenase and peroxidase active sites. With an 87% identification rate and a strict sequence, conservation in the cyclooxygenase active site, the structure of human COX-2 is expected to be very similar to the murine enzyme.

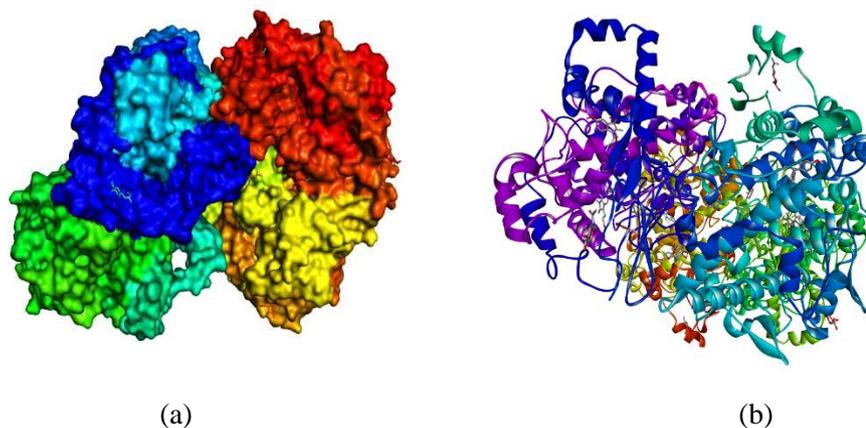


Figure 1. The 4COX protein preparation by *Biovia Discovery Studio software*

Validation of docking method

The redocking method with Autodock-4 was used to validate the docking process, specifically on the active side of the co-crystal ligand, indomethacin. The RMSD value from the redocking results was 0.78 Å, which was less than 3.00 Å. As shown in Table 1. This result was indicating that the conformation of the ligand from the redocking results not differ significantly from the crystallographic results shown in Figure 3. The native ligand, indomethacin, a tested compounds, Sulphated polysaccharides, and diclofenac sodium as controls were visualized in 2D using Chem draw ultra software. Geometry optimization was carried out by using Avogadro software and energy minimization using Chem 3D software with a semi-empirical method (MM2) in order to obtain a better and more stable structural conformation as shown in Figure 2.

Table 1. The grid box ligand in the COX2 as used in redocking validation method by *Autodock*

Ligand	Grid Point			Coordinate Grid Point			Grid Resolution	RMSD
	X	Y	Z	X	Y	Z		
Native Ligan	X	Y	Z	X	Y	Z		
<i>Indomethacin</i>	40	40	40	60.804	44.603	72.176	0.375 A	0.78 A

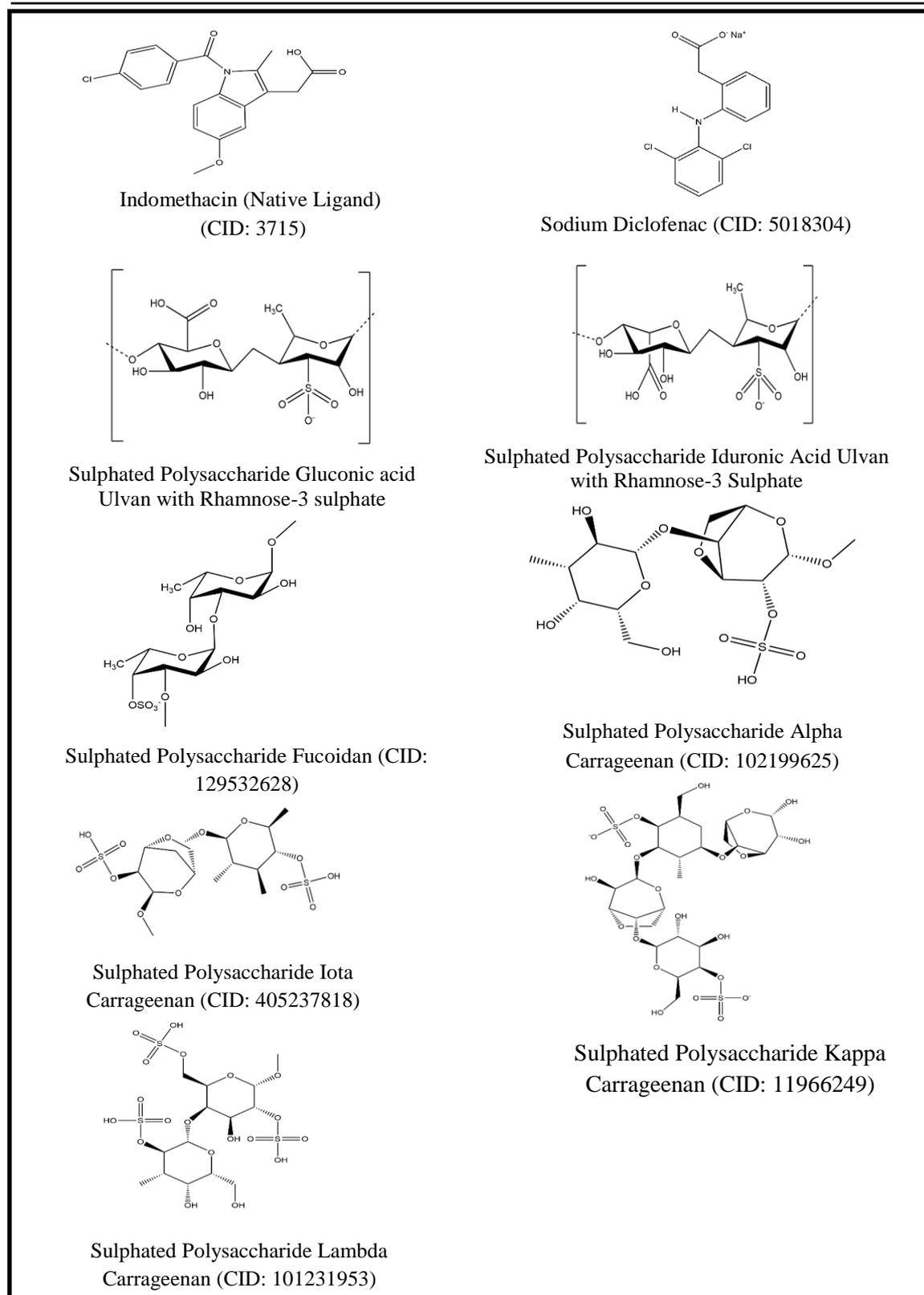


Figure 2. Two-dimensional chemical structure of selected compounds (ligands) retrieved from PubChem database with compound ID

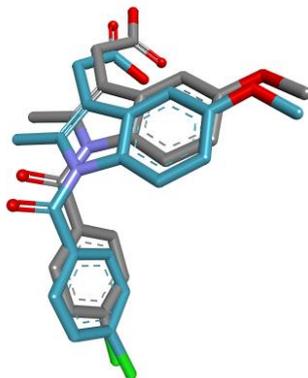


Figure 3. Validation properties of native ligand from docked structure (blue color) and crystallographic structure (grey color)

Molecular docking analysis

In this study, totally nine compounds were subjected to the molecular docking study. Among them, six compounds were sulphated polysaccharide, one was indomethacin as native ligand and one commercial anti-inflammatory drugs, namely sodium diclofenac were docked with 4COX. The binding affinity and Inhibition constant (IC) were used as the parameter to evaluate the interactions of ligands with 4COX. [Table 2](#) showed the summary of docking result including the two- and three-dimensional molecular interactions of protein–ligand complexes. Sodium diclofenac was used as positive control anti-inflammatory drugs due to the wide usage in Indonesia. Moreover, as shown in [Table 2](#) five sulphated polysaccharides performed the binding affinity energy equal to sodium diclofenac. The best binding affinity was obtained by *Gluconic acid ulvan* -7.62 kcal/mol slightly higher to the commercial anti-inflammatory drug sodium diclofenac (-7.81 kcal/mol), followed by *Iduronic acid Ulvan* -7.57 kcal/mol, *Fucoidan* (-6.11 kcal/mol), *Alpha Carrageenan* (-6.93 kcal/mol), and *Lambda Carrageenan* (-5.38 kcal/mol). The best binding affinity was obtained in *Gluconic acid ulvan* with binding affinity -7.65 kcal/mol with the lowest inhibition constant of 2.58 μM . The others four sulphated polysaccharides had higher inhibition constant around 2.83 μM up to 114.02 μM . Overall, the molecular docking analysis was presented the potency of sulphated polysaccharides as 4COX antagonist.

[Table 2](#) and [Figure 4](#), [Figure 5](#), [Figure 6](#) presents a detailed analysis of ligand-protein interactions in two and three dimensions. Indomethacin, a non-selective cyclooxygenase inhibitor, binds to the enzyme's active site. Indomethacin penetrates the hydrophobic channel the most of the complexes. It contains a chlorine atom that interacts with the side-chain conformation of Leu 384. The benzoyl group occupies an environment formed in the stable formation via hydrophobic interaction with Leu 384, Phe 381, Tyr 385, and Trp 387. The benzoyl oxygen reacts with the hydroxyl Ser 530 side chain and the Val 349 side chain ([Kurumbail et al., 1996](#)). The carboxylate forms a salt bridge with Arg 120, and the indole ring interacts with Val 349 and Ser 353. Tyr 355, Val 523, and Ala 527 are further contacted. The six-membered ring of indole interacts strongly with the main-chain atoms of Leu 352 and Ser 353. The o-methoxy group of indomethacin protrudes slightly into a relatively large cavity created by COX-2 near Ser 353, Tyr 355, and Val 523. As a result, indomethacin's binding affinity with COX2 was very low (-11.05 kcal/mol), indicating that it has a high affinity for 4COX.

Table 2. Binding energy, inhibition concentration (Ki), and interactive residue of native ligand, commercial anti-inflammatory drugs: Indomethacin, and sulphated polysaccharide docked against 4COX receptor

Pubchem ID	Compound name	Binding energy (kcal/mol)	Ki	Interactive residue
3715	<i>Indomethacin</i>	-11.05 kcal/mol	8.01 nM	Ser530, Ser 353, Met 522, Val349, Tyr385, Trp387, Val349, Leu 352, Leu384, Val523, Ala527, Leu531, Trp387.
5018304	<i>Sodium diclofenac</i>	-7.81 kcal/mol	1.89 uM	Ser530, Val523, Ala527, Gly526, Tyr385, Val349, Leu384, Leu352, Met522, Val523, Ala527.
	<i>Gluconic acid ulvan</i>	-7.62 kcal/mol	2.58 uM	Arg120, Arg120, Tyr385, Ser530, Tyr385, Ser530, Val349, Leu352, Tyr355, Leu359.
	<i>Iduronic acid Ulvan</i>	-7.57 kcal/mol	2.83 uM	Arg120, Tyr385, Met522, Ser530, Ser530, Val349, Leu352, Tyr355, Leu359, Val523, Ala527.
129532628	<i>Fucoidan</i>	-6.11 kcal/mol	33.32 μM	Arg120, Tyr385, Met522, Ser530, Arg120, Ser530, Tyr385, Tyr348, Tyr355, Val523.
102199625	<i>Alpha Carrageenan</i>	-6.93 kcal/mol	8.35 μM	Arg120, Tyr355, Tyr385, Arg120, Tyr385, Tyr348, Val523, Tyr355.
405237818	<i>Iota Carrageenan</i>	-7,56 Kcal/mol	2,88 μM	Tyr348, Tyr385, Ala527, Ser530, Leu384, Met522, Val523, Arg120, Tyr348, Trp387, Val349, Tyr355, Val523, Ala527, Leu531.
11966249	<i>Kappa Carrageenan</i>	-2.35 kcal/mol	19.02 mM	Arg120, Arg120, Tyr355, Met522, Ser530, Tyr385, Met522.
11966249	<i>Lambda Carrageenan</i>	-5.38 kcal/mol	114.02 μM	Arg120, Ser353, Tyr355, Ala527, Leu531, His90, Tyr 355, Val523, Ser530, His90, Leu352, Tyr385, Trp387.

Noted : **Green**: Hydrogen Bond ; **Violet**: Pi-Sigma Bond ; **Pink**: Pi-Pi Stacked ; **Light pink**: Pi-Alkyl Bond ; **Red**: Unfavorable bond ; **Yellow**: Pi-Sulphur Bond ; **Orange**: Attractive Charge Bond ; **Blue**: Halogen Bond ; **Cyan**: Carbon-Hydrogen Bond

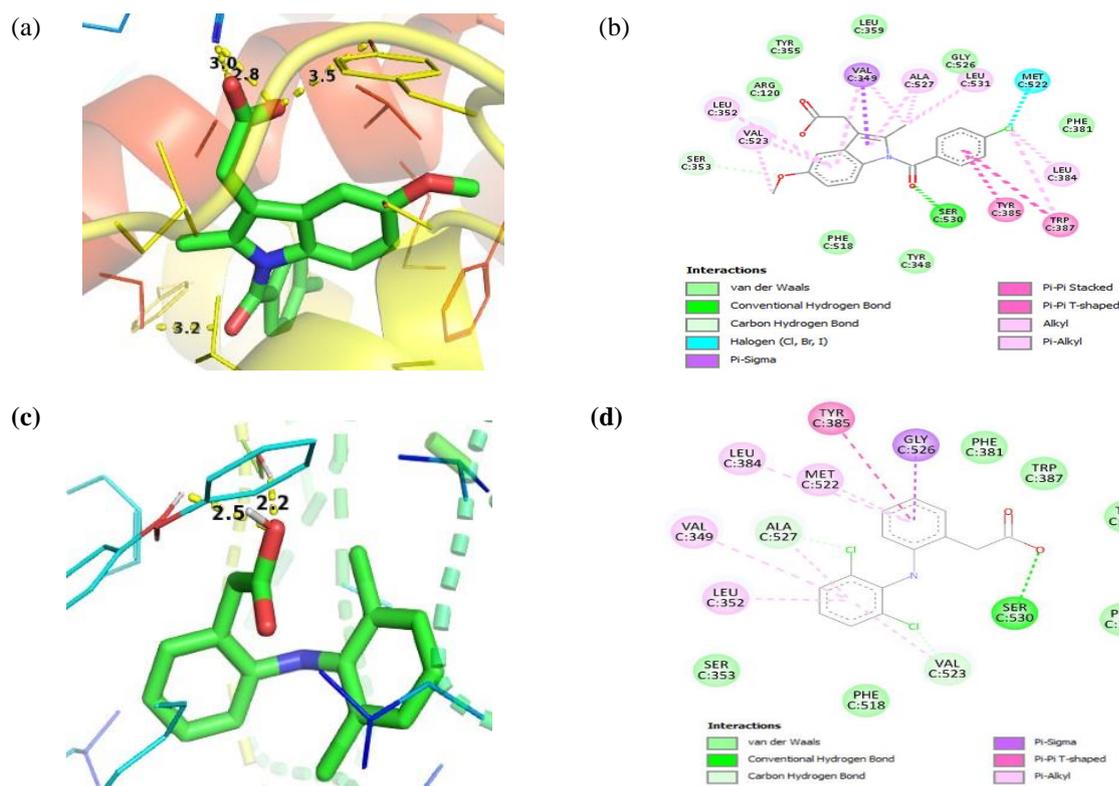


Figure 4. Molecular interactions of 4COX receptor with Indometazin (native ligand) 3D interaction (a) and 2D interaction (b); Sodium diclofenac 3D interaction (c) and 2D interaction (d)

Diclofenac demonstrated hydrogen binding of the carboxyl-Cl-H with the OH-groups of Tyr 385, Val 523, and Val 349, as well as alkyl interaction with Leu 531, Tyr 348, and Leu 352. Indomethacin, sodium diclofenac, and three ulvans, gluconic acid ulvan, iodoronic acid ulvan, and Fucoidan, the amino acids Ser 530 and Tyr 385 appear to be important binding sites that formed hydrogen bonds. Tyr-385 and Ser-530 were identified as residues that can collaborate in the chelation of negative charges or electron-rich centers due to their unique orientation. Tyr-385 hydrogen bonding is proposed to stabilize the negative charge of the tetrahedral intermediate formed during Ser-530 acetylation (Rowlinson et al., 2003). This pattern of interaction is in line with studies of the interaction of COX2 receptors with several anti-inflammatory drugs. The derivatives of 2-mercapto-4(3H)-quinazolinones showed the H-bonding in the same amino acid: the N-pyridine performed proper H-bonding with His90, the crucial key amino acid at the active site. The carbonyl oxygen formed bifurcated H-bonds with Tyr385 and Ser530, whereas the N-hydrazino terminal function enriched the pocket with the donor-acceptor interaction and was held by trifurcated strong H-bonds with Gly526, Ala527, and Ser530 (Abdel-Aziz et al., 2016). Ruslin et al. (2022) was also stated that naproxen, the well-known anti-inflammatory agent showed one hydrogen bonding interaction with Tyr355 and hydrophobic interactions with Ala527, Gly526, Trp387, Tyr385, and Leu352.

In the last, based on *in silico* study, sulphated polysaccharides belongs to *Ulva lactuca* L, L in both of ulvan and carrageenan compounds had similar interaction with COX2 to sodium diclofenac as commercial anti-inflammatory drug. Thus, further *in vitro* and *in vivo* studies can be looked upon to take these bioactive sulphated polysaccharides to the next level of application in anti-inflammatory treatment.

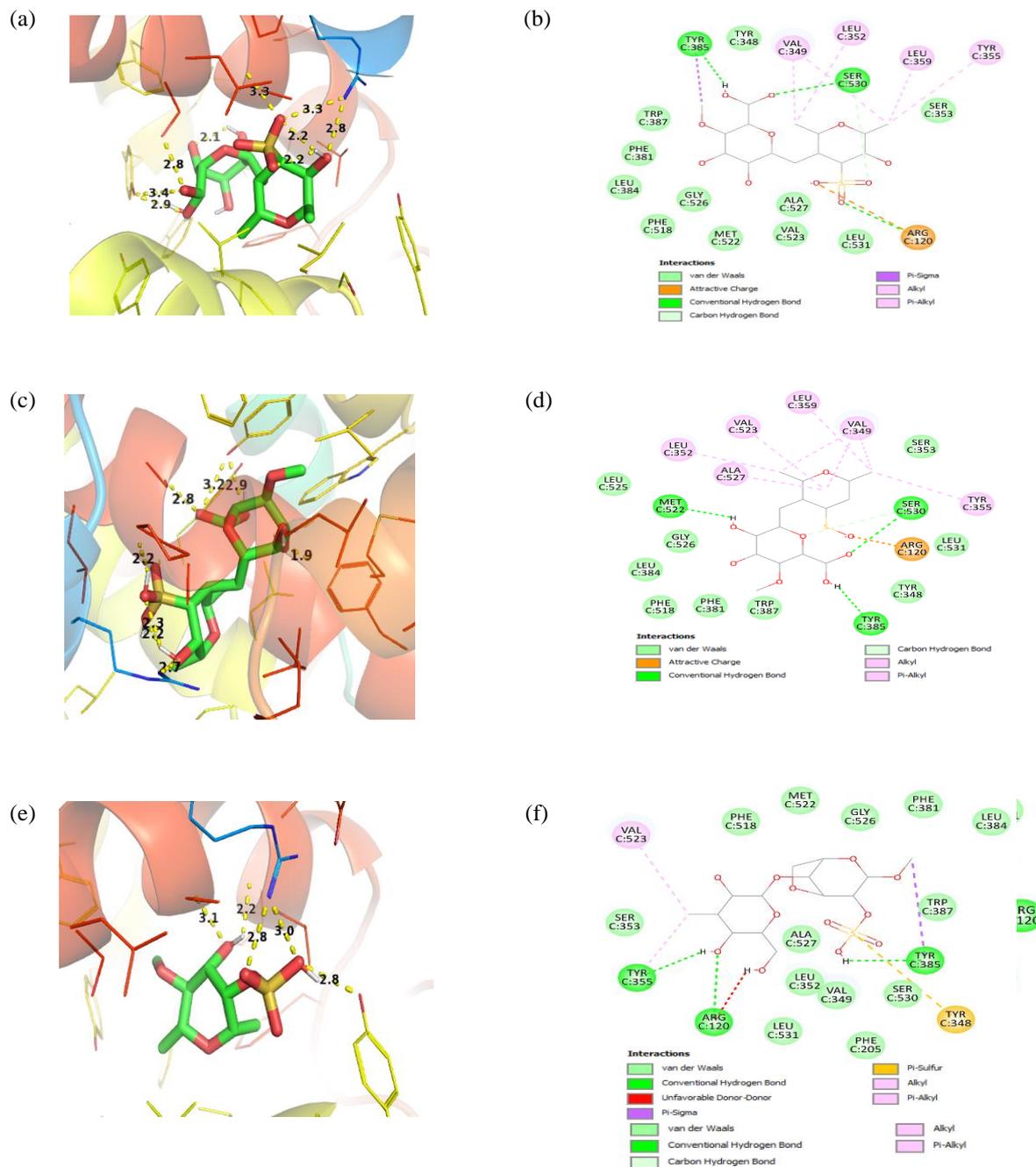


Figure 5. Molecular interactions of 4COX receptor with *Gluconic acid ulvan* 3D interaction (a) and 2D interaction (b); *Iduronic acid Ulvan* 3D interaction (c) and 2D interaction (d); *Fucoidan* 3D interaction (e) and 2D interaction (f)

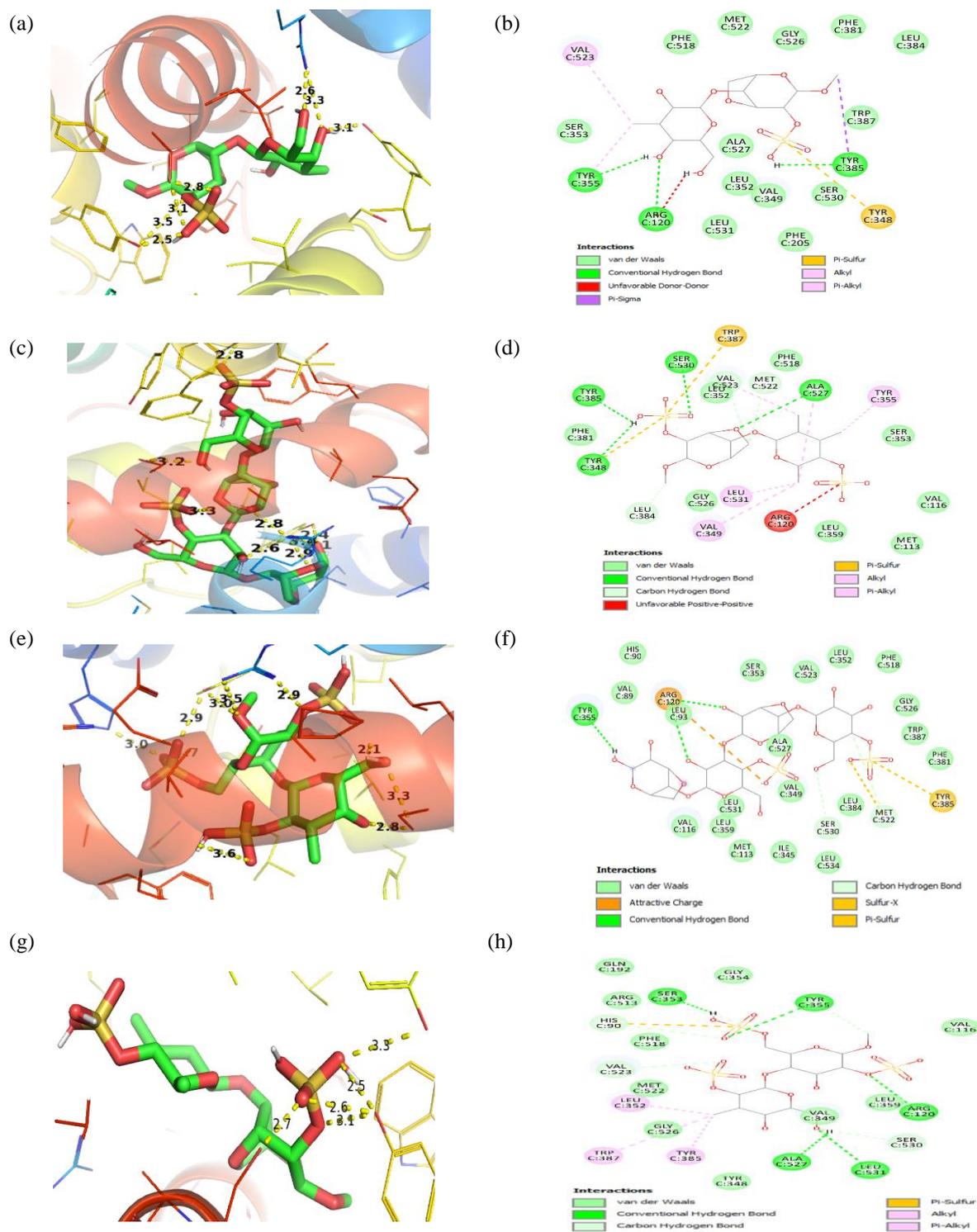


Figure 6. Molecular interactions of 4COX receptor with carrageenan alpha 3D interaction (a) and 2D interaction (b); carrageenan Iota 3D interaction (c) and 2D interaction (d); carrageenan Kappa I 3D interaction (e) and 2D interaction (f); carrageenan lambda 3D interaction (g) and 2D interaction (h)

CONCLUSION

The exploration of sulphated polysaccharide as the rare polysaccharide chemical constituent of green algae becomes arise in the last decade. In this context, the docking molecular for virtual screening of the natural sulfated of green algae (*Ulva lactuca L*) were docked with the important enzyme in inflammatory process (COX2) to investigate the ability of the compounds to inhibit the COX2 receptor activity. The docking results attained strongly suggest that the sulphated polysaccharide in *Ulva lactuca L* had shown an interaction with COX2 similiar to commercial leading drugs used for inflammation sodium diclofenac. Furthermore, among the all sulphated polysaccharide compounds, *Gluconic acid ulvan* showed the best binding afnity with H-bond interactions. Therefore, based on the mechanism of interaction of these ligands with the 4COX targeted enzyme, it needs to be further evaluated by conducting *in vitro* studies to confrm their anti-inflammatory activity of aqueous extract of *Ulva lactuca L*.

ACKNOWLEDGEMENT

The authors thanks to the Institute of Research and Community Service (LPPM) of Universitas Ahmad Dahlan for the funding in regular research funding with the grand number: PD-022/SP3/LPPM-UAD/VII/2022. Special thanks to Ryan Syahputra in 2D visualization of tested compound visualization.

REFERENCES

- Abdel-Aziz, A. A.-M., Abou-Zeid, L. A., ElTahir, K. E. H., Ayyad, R. R., El-Sayed, M. A.-A., & El-Azab, A. S. (2016). Synthesis, anti-inflammatory, analgesic, COX-1/2 inhibitory activities and molecular docking studies of substituted 2-mercapto-4(3H)-quinazolinones. *European Journal of Medicinal Chemistry*, *121*, 410–421. <https://doi.org/10.1016/j.ejmech.2016.05.066>.
- Ahmadi, A., Zorofchian Moghadamtousi, S., Abubakar, S., & Zandi, K. (2015). Antiviral potential of Algae polysaccharides isolated from Marine Sources: a review. *BioMed Research International*, *2015*, 1–10. <https://doi.org/10.1155/2015/825203>.
- Bernardi, P., Rasola, A., Forte, M., & Lippe, G. (2015). The mitochondrial permeability transition pore: channel formation by F-ATP synthase, integration in signal transduction, and role in pathophysiology. *Physiological Reviews*, *95*(4), 1111–1155. <https://doi.org/10.1152/physrev.00001.2015>.
- Cindana Mo'o, F. R., Wilar, G., Devkota, H. P., & Wathoni, N. (2020). Ulvan, a polysaccharide from Macroalga *Ulva* sp.: A review of chemistry, Biological activities and potential for food and biomedical applications. *Applied Sciences*, *10*(16), 5488. <https://doi.org/10.3390/app10165488>.
- Jain, A. N., & Nicholls, A. (2008). Recommendations for evaluation of computational methods. *Journal of Computer-Aided Molecular Design*, *22*(3–4), 133–139. <https://doi.org/10.1007/s10822-008-9196-5>.
- Jiao, G., Yu, G., Zhang, J., & Ewart, H. (2011). Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Marine Drugs*, *9*(2), 196–223. <https://doi.org/10.3390/md9020196>.
- Jin, H., Zhang, Z., Wang, C., Tang, Q., Wang, J., Bai, X., Wang, Q., Nisar, M., Tian, N., Wang, Q., Mao, C., Zhang, X., & Wang, X. (2018). Melatonin protects endothelial progenitor cells against AGE-induced apoptosis via autophagy flux stimulation and promotes wound healing in diabetic mice. *Experimental & Molecular Medicine*, *50*(11), 1–15. <https://doi.org/10.1038/s12276-018-0177-z>.
- Kepel, R. C., Lumingas, L. J. L., Watung, P. M. M., & Mantir, D. M. H. (2019). Community structure of seaweeds along the intertidal zone of Mantehage Island, North Sulawesi, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society*, *12*(1), 87–101. www.bioflux.com.ro/aacl.
- Kidgell, J. T., Magnusson, M., de Nys, R., & Glasson, C. R. K. (2019). Ulvan: a systematic review of extraction, composition and function. *Algal Research*, *39*, 101422.

- <https://doi.org/10.1016/j.algal.2019.101422>.
- Kim, J.-K., Cho, M. L., Karnjanapratum, S., Shin, I.-S., & You, S. G. (2011). In vitro and in vivo immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*. *International Journal of Biological Macromolecules*, 49(5), 1051–1058. <https://doi.org/10.1016/j.ijbiomac.2011.08.032>.
- Kim, M.-J., Kim, K.-B.-W.-R., Jeong, D.-H., & Ahn, D.-H. (2013). Inhibitory effect of *Sargassum fulvellum* ethanolic extract on LPS-induced inflammatory reaction in RAW 264.7 mouse macrophages. *J Appl Biol Chem*, 56(4), 249–255.
- Kontoyianni, M., McClellan, L. M., & Sokol, G. S. (2004). Evaluation of docking performance: comparative data on docking algorithms. *Journal of Medicinal Chemistry*, 47(3), 558–565. <https://doi.org/10.1021/jm0302997>.
- Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J. J., Stegeman, R. A., Pak, J. Y., Gildehaus, D., Iyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C., & Stallings, W. C. (1996). Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, 384(6610), 644–648. <https://doi.org/10.1038/384644a0>.
- Lakshmi, D. S., Sankaranarayanan, S., Gajaria, T. K., Li, G., Kujawski, W., Kujawa, J., & Navia, R. (2020). A short review on the valorization of green seaweeds and ulvan: feedstock for chemicals and biomaterials. *Biomolecules*, 10(7), 991. <https://doi.org/10.3390/biom10070991>.
- Leiro, J. M., Varela, M., Piazzon, M. C., Arranz, J. A., Noya, M., & Lamas, J. (2010). The anti-inflammatory activity of the polyphenol resveratrol may be partially related to inhibition of tumour necrosis factor- α (TNF- α) pre-mRNA splicing. *Molecular Immunology*, 47(5), 1114–1120. <https://doi.org/10.1016/j.molimm.2009.10.030>.
- Li, S., & Shah, N. P. (2014). Antioxidant and antibacterial activities of sulphated polysaccharides from *Pleurotus eryngii* and *Streptococcus thermophilus* ASCC 1275. *Food Chemistry*, 165, 262–270. <https://doi.org/10.1016/j.foodchem.2014.05.110>.
- Liu, T., Li, Q., Xu, X., Li, G., Tian, C., & Zhang, T. (2022). Molecular mechanisms of anti-cancer bioactivities of seaweed polysaccharides. *Chinese Herbal Medicines*, 14(4), 528–534. <https://doi.org/10.1016/j.chmed.2022.02.003>.
- Motoyama, K., Tanida, Y., Sakai, A., Higashi, T., Kaneko, S., & Arima, H. (2018). Anti-allergic effects of novel sulfated polysaccharide sacran on mouse model of 2,4-Dinitro-1-fluorobenzene-induced atopic dermatitis. *International Journal of Biological Macromolecules*, 108, 112–118. <https://doi.org/10.1016/j.ijbiomac.2017.11.155>.
- Novianti, A. D., Karang, I. W. G. A., & Putra, I. N. G. (2020). Optimalisasi Biomassa Alga Hijau *Ulva* sp. sebagai Biosorben logam berat Cr(VI). *Journal of Marine and Aquatic Sciences*, 6(1), 125. <https://doi.org/10.24843/jmas.2020.v06.i01.p15>.
- Peasura, N., Laohakunjit, N., Kerchoechuen, O., Vongsawadi, P., & Chao, L. K. (2016). Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from *Ulva intestinalis*. *International Journal of Biological Macromolecules*, 91, 269–277. <https://doi.org/10.1016/j.ijbiomac.2016.05.062>.
- Reiter, R. J., Mayo, J. C., Tan, D.-X., Sainz, R. M., Alatorre-Jimenez, M., & Qin, L. (2016). Melatonin as an antioxidant: under promises but over delivers. *Journal of Pineal Research*, 61(3), 253–278. <https://doi.org/10.1111/jpi.12360>.
- Rowlinson, S. W., Kiefer, J. R., Prusakiewicz, J. J., Pawlitz, J. L., Kozak, K. R., Kalgutkar, A. S., Stallings, W. C., Kurumbail, R. G., & Marnett, L. J. (2003). A novel mechanism of cyclooxygenase-2 inhibition involving interactions with Ser-530 and Tyr-385. *Journal of Biological Chemistry*, 278(46), 45763–45769. <https://doi.org/10.1074/jbc.M305481200>.
- Ruslin, R., Yamin, Y., Kasmawati, H., Mangrura, S., Kadidae, L., Armid, A., & Arba, M. (2022). The search for Cyclooxygenase-2 (COX-2) inhibitors for the treatment of inflammation disease: an in-silico study. *Journal of Multidisciplinary Healthcare, Volume 15*, 783–791. <https://doi.org/10.2147/JMDH.S359429>.
- Tabarsa, M., You, S., Dabaghian, E. H., & Surayot, U. (2018). Water-soluble polysaccharides from

- Ulva intestinalis: Molecular properties, structural elucidation and immunomodulatory activities. *Journal of Food and Drug Analysis*, 26(2), 599–608. <https://doi.org/10.1016/j.jfda.2017.07.016>.
- Tang, Y., Rosenberg, J. N., Bohatskyi, P., Yu, G., Betenbaugh, M. J., & Wang, F. (2016). Microalgae as a feedstock for Biofuel precursors and value-added products: green fuels and golden opportunities. *Bioresource.Com*, 11(1), 2850–2885.
- Tziveleka, L.-A., Pippa, N., Georgantea, P., Ioannou, E., Demetzos, C., & Roussis, V. (2018). Marine sulfated polysaccharides as versatile polyelectrolytes for the development of drug delivery nanoplatforms: Complexation of ulvan with lysozyme. *International Journal of Biological Macromolecules*, 118, 69–75. <https://doi.org/10.1016/j.ijbiomac.2018.06.050>.
- Vasconcelos, A. F. D., Dekker, R. F. H., Barbosa, A. M., Carbonero, E. R., Silveira, J. L. M., Glauser, B., Pereira, M. S., & Corradi da Silva, M. de L. (2013). Sulfonation and anticoagulant activity of fungal exocellular β -(1→6)-d-glucan (lasiodiplodan). *Carbohydrate Polymers*, 92(2), 1908–1914. <https://doi.org/10.1016/j.carbpol.2012.10.034>.
- Yu-Qing, T., Mahmood, K., Shehzadi, R., & Ashraf, M. F. (2016). Ulva lactuca and Its Polysaccharides: Food and Biomedical Aspects. *Journal of Biology, Agriculture and Healthcare*, 6(1), 140–151.